Luteal Adenylyl Cyclase Does Not Develop Sensitivity to Desensitization by Human Chorionic Gonadotropin in the Absence of Nonluteal Ovarian Tissue*

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ABSTRACT. There is evidence suggesting that the mere presence of a hormone-responsive adenylyl cyclase system in a tissue may not be sufficient for desensitization to occur since phosphorylation reactions might also be involved. The purpose of this study was to determine if luteal tissue in the absence of other ovarian tissues would desensitize to human CG (hCG). One or both ovaries were removed from rabbits 5 h before hCG-induced ovulation and the periovulatory follicles were transplanted underneath the kidney capsule where they formed ectopic corpus luteum [or corpora lutea (CL)]. Rabbits which were bilaterally ovariectomized received estradiol implants at the time of ovariectomy to maintain control serum estradiol concentrations. On day 7 of pseudopregnancy, the rabbits were injected with saline (control) or with 75 IU hCG and were killed 24 h later at which time ovarian and ectopic CL progesterone content and adenylyl cyclase activity were assessed. As expected, in ovarian CL there was decreased LH-responsive adenylyl cyclase (69% relative to

control) and a correspondingly decreased luteal progesterone content (40% relative to control). In the same rabbits, the ectopic CL showed much the same pattern of response as the ovarian CL but perhaps to a slightly lesser extent (decreases relative to control of 59% in adenylyl cyclase response to LH and 29% in progesterone content). However, in rabbits with ectopic CL only, the luteal tissue showed no change either in hormone-responsive adenylyl cyclase activity or in progesterone content. Similarly, binding of radiolabeled hCG to luteal membranes 24 h after hCG was almost totally absent in ovarian CL, was decreased by 50% in ectopic CL with one ovary present, and was unaltered in ectopic CL of bilaterally ovariectomized rabbits. These data suggest that nonluteal ovarian tissue may be required for the induction in CL of the appropriate protein kinases for the proposed phosphorylations involved in adenylyl cyclase desensitization. (*Endocrinology* **113**: 2052, 1983)

BESIDES being the impetus for ovulation and subsequent corpus luteum [or corpora lutea (CL)] formation, the ovulatory LH surge serves to cause steroidogenic quiescence in antral follicles after a short lived burst of steroidogenic activity. This has been documented in several species including the rabbit (1–14) and the rat (5–7). The steroidogenic quiescence associated with ovulation is preceded by desensitization of the gonadotropin-responsive adenylyl cyclase of the preovulatory follicles (8, 9) and adenylyl cyclase in newly formed CL is hormone insensitive (9, 10). As a result, gonadotropin-induced desensitization of antral follicles at the time of the ovulatory surge would appear to prevent runaway steroidogenesis in response to the highly elevated serum concentration of LH and seems to subserve a physiological role.

There may also be a physiological role for gonadotropin-mediated desensitization of luteal adenylyl cyclase. In mice, pregnant females will fail to implant and will

resume cyclicity if exposed to a strange male (11). This phenomenon, known as the Bruce effect, is characterized by olfactory-induced pituitary gonadotropin release (12) which could serve to desensitize the CL adenylyl cyclase and to initiate luteolysis with concomitant failure of implantation, and resumption of the estrous cycle.

Gonadotropin-induced desensitization has been studied in ovarian follicular and luteal tissues of a number of species [see Birnbaumer and Kirchick, (13) for review], and it has been found that not all ovarian adenylyl cyclases are sensitive to desensitization by their stimulatory gonadotropins. Notably, it has been reported recently that preantral follicles are resistant to FSH-induced desensitization despite the presence of a FSH-responsive adenylyl cyclase in this tissue type (14). This lack of desensitization has been proposed (14) to be what allows preantral follicles to survive the ovulatory gonadotropin surge and continue development to the antral stage. It follows therefore that the capacity of a hormone-sensitive adenylyl cyclase system to respond in a desensitizing manner to its stimulatory hormone is either a property that needs to develop and can do so independently of a development of its sensitivity to respond

, positively to the hormonal stimulus, or is a function that can be inhibited or suppressed by as yet undetermined factors present in preantral but absent in antral follicles.

Ectopic CL is obtained upon transplantation of human CG (hCG)-stimulated preovulatory follicles underneath the kidney capsule. Studying factors that affect rabbit CL life span by comparing progesterone content and responsiveness of adenylyl cyclase to stimulatory hormones in normal in situ (ovarian) CL to those of ectopic CL, we noticed that ectopic CL in ovariectomized rabbits develop the capacity to be stimulated by LH and hCG but lack the capacity to be desensitized by hCG. This finding constitutes the body of this communication.

Materials and Methods

Materials

Inorganic ^{32}P was purchased from CintiChem, Inc. (Tuxedo, NY), ^{125}I (carrier-free) was obtained from Iso-Tex Diagnostics (Friendswood, TX), and $[^3H]cAMP$ was purchased from Schwarz/Mann (Orangeburg, NY). Xylazine (Rompun) from Cutter Laboratories, Inc., Shawnee, KS) and ketamine HCl (Ketaset from Bristol Laboratories, Syracuse, NY) were used as anesthetics. hCG for injection was from Ayerst Laboratories (New York, NY). Highly purified hCG for iodination (hCG-CR119) and LH (NIH-LH-B9) were obtained from the NIH. (−)Isoproterenol was a gift from the Sterling Winthrop Research Institute (Rensselaer, NY). Progesterone and estradiol (E₂) derivatives for iodination were steroid-11-hemisuccinate-tyrosine-methyl-esters (steroid-11-TME) and were gifts from Dr. John E. Pike (Upjohn Co., Kalamazoo, MI). Creatine phosphate and creatine kinase were obtained from Calbiochem (La Jolla, CA); myokinase, ATP (Tris-salt), guanosine triphosphate, cAMP, EDTA, NaF, and Tris were purchased from Sigma Chemical Co. (St. Louis, MO); $[\alpha\text{-}^{32}P]ATP$ (400–600 Ci/mmol), synthesized from ^{32}P according to the procedure of Walseth and Johnson (15) and purified according to the method of Birnbaumer et al. (16), was supplied by the Core Laboratory for Cyclic Nucleotide Research, Center for Population Research and Studies on Reproductive Biology, Baylor College of Medicine (Houston, TX).

E₂-filled Silastic capsules (id, 1.57 mm; od, 2.42 mm) were prepared according to the method of Legan et al. (17), except that the total length of the capsule was 9 mm, with a 3-mm fill length. $[^{125}I]Iodo\text{-}hCG$ was prepared using the lactoperoxidase procedure developed for LH (18) as modified for hCG (19).

Animals and treatments

Virgin New Zealand white rabbits (3–4 kg) were housed in individual cages in air-conditioned quarters and were fed ad libitum Purina rabbit chow for at least 15 days before the initiation of experiments. Pseudopregnancy was induced by the iv injection of 75 IU hCG. About 5 h before expected ovulation, rabbits were anesthetized by im injection of 5 mg/kg Xylazine followed about 5 min later with 35 mg/kg ketamine HCl. One (Fig. 1; Protocol A) or both (Fig. 1; Protocol B) ovaries were removed, periovulatory follicles were dissected free from one of
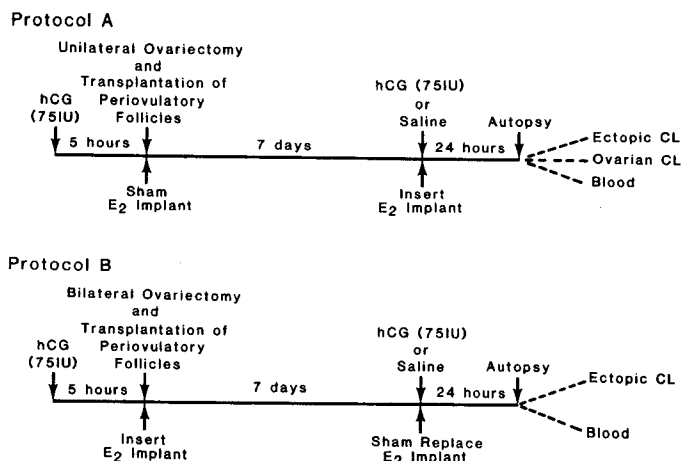

FIG. 1. Experimental protocols used to test the effects of a desensitizing dose of hCG (75 IU) on ectopic CL. Protocol A results in four sets of CL: 1) ovarian CL from saline-injected rabbits, 2) ectopic CL from the same rabbits as in 1, 3) ovarian CL from hCG-injected rabbits, and 4) ectopic CL from the same rabbits as in 3. Protocol B results in two additional sets of CL: 5) ectopic CL from saline-injected rabbits with no extraluteal ovarian tissue, and 6) ectopic CL from hCG-injected rabbits with no extraluteal ovarian tissue.

the excised ovaries, and the follicles were injected underneath the kidney capsule as described by Keyes and Armstrong (20). Such transplanted follicles develop into ectopic CL. Animals which had been bilaterally ovariectomized were implanted sc with an E₂-filled Silastic capsule and unilaterally ovariectomized rabbits were implanted with empty capsules at the time of ovariectomy. The day after hCG injection was designated day 1 of pseudopregnancy.

On day 7 of pseudopregnancy, rabbits received either saline (control) or 75 IU hCG iv. The empty capsules were replaced with E₂-filled capsules in the unilaterally ovariectomized rabbits without anesthesia immediately after the saline or hCG injections. Sham replacement was performed in bilaterally ovariectomized rabbits. All rabbits were killed by cervical dislocation 24 h later. As summarized in Fig. 1, six different groups of CL were thus obtained from the two protocols: 1) ovarian (in situ) CL from saline injected rabbits; 2) ectopic CL from the same rabbits as in 1); 3) ovarian CL from hCG injected rabbits; 4) ectopic CL from the same rabbits as in 3); 5) ectopic CL from bilaterally ovariectomized saline injected rabbits, and 6) ectopic CL from bilaterally ovariectomized hCG injected hCG injected rabbits. At the time of killing, blood was collected by cardiac puncture and the ovary (if present) and ectopic CL were removed and placed in ice-cold Krebs-Ringer bicarbonate buffer prepared with half the recommended CaCl₂ (21).

Preparation of sera and CL homogenates and membranes

After collection, the blood was allowed to stand at room temperature for 30 min and for 4 h at 4 C. Sera were obtained after centrifugation in a refrigerated centrifuge and were stored at −20 C until assayed for E₂ by RIA (see below).

Within 1 h of killing, ovarian CL were dissected free of the ovaries, cleaned of adhering interstitial tissue, and were blotted and weighed. Ectopic CL were dissected free of the kidney

capsule membrane, cleaned of adhering tissues, and were blotted and weighed as well. The luteal tissues thus obtained were either homogenized and used immediately for adenylyl cyclase assays or were further processed to obtain membrane particle preparations as described previously (22) which were quick-frozen and stored at −70 C until assayed for hormone receptor levels (see below). A small portion of each homogenate was aliquoted, quick-frozen, and stored at −20 C for tissue progesterone analysis at a later time (see below).

Adenylyl cyclase assays

Adenylyl cyclase activity in 20-μl aliquots of homogenates was determined as described earlier (9). When present, the concentrations of LH, isoproterenol, and NaF were 10 μg/ml, 100 μM, and 10 mM, respectively. The [^{32}P]cAMP formed was quantitated by the method of Salomon et al. (23), as modified by Bockaert et al. (24). Protein was determined by the method of Lowry et al. (25) using BSA (fraction V, Armour Pharmaceutical Co., Chicago, IL) as standard.

[^{125}I]Iodo-hCG Binding Assays

Available LH/hCG receptors were assessed by a binding assay described previously (19) using about 10 μg membrane protein in the presence of up to 5 nM [^{125}I]iodo-hCG (1.2 × 10^6 cpm), 25 mM Tris-HCl (pH 7.5), 1 mM EDTA, and 1% BSA in 100 μl. The reactions were stopped and bound labeled ligand was separated from free by a variation of the polyethylene glycol precipitation method described recently (26). Nonspecific binding was measured in the presence of 10 μg/ml LH. All points were assayed in triplicate and the data were subjected to Scatchard analysis to determine the dissociation constant (K_d) and the maximum binding (B_{max}).

RIAs for E_2 and progesterone

Serum E_2 and luteal progesterone were assessed by RIAs as described elsewhere (27). Modifications described by Goodman et al. (28) were employed for the E_2 RIA which used GDN 930 (supplied by Dr. P. Landis Keyes, University of Michigan, Ann Arbor, MI) at a final dilution of 1:1,155,000 as the antiserum. The labeled ligand was [^{125}I]iodo-E_2-11-TME. For the progesterone assays, GDN 337 (supplied by Dr. Gordon D. Niswender, Colorado State University, Fort Collins, CO) was used as the antiserum at a final dilution of 1:320,000 and the labeled ligand was [^{125}I]iodo-progesterone-11-TME. In these assays, nonspecific binding, percent binding, 50% inhibition, sensitivity, slope of the standard curve, and interassay variation were essentially the same as reported previously (27) with the following exception: the 50% inhibition point for the E_2 assay was 4.1 ± 0.2 pg/tube.

Statistics

RIA data were analyzed using a computer program based on the assay statistics described by Midgley et al. (29) and Duddleson et al. (30). The Scatchard analyses employed simple linear regression since the correlation coefficients were all 0.98 in assays where receptor levels were measurable. Comparisons between group means were performed using analysis of variance.

Results

Uptake of radiolabeled hCG by ovarian vs. ectopic CL

It was necessary to determine whether injected hCG could reach and be retained by ectopic CL. Therefore, 40 μCi [^{125}I]iodo-hCG were injected into four rabbits which had both ectopic and ovarian CL. CL removed 2 h after the injection were weighed and the amount of radioactivity present was determined. The results of this experiment are shown in Fig. 2. There was no difference in [^{125}I]iodo-hCG uptake between ectopic and ovarian CL. The counts present in renal tissue are also shown and represent nonspecific uptake of radiolabeled hormone.

Serum E_2 concentrations

Since half of the rabbits in this study were bilaterally ovariectomized, E_2-filled capsules were used in such animals in order to maintain the ectopic CL. These E_2-filled capsules resulted in serum E_2 concentrations of 3.2 ± 0.9 pg/ml (mean ± SEM) compared to 3.4 ± 0.8 pg/ml in untreated pseudopregnant rabbits (27). Serum E_2 levels in unilaterally ovariectomized rabbits 24 h after receiving the E_2-filled capsule (see Materials and Methods and Fig. 1) were 3.7 ± 0.8 pg/ml.
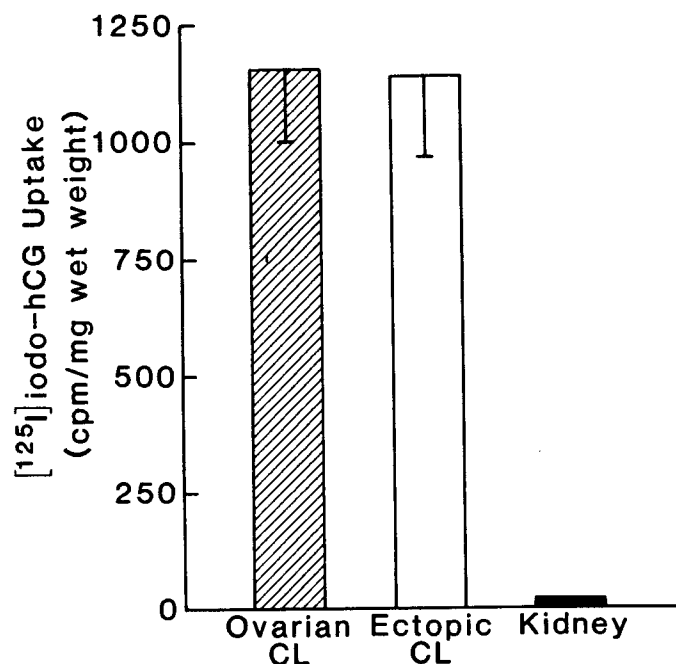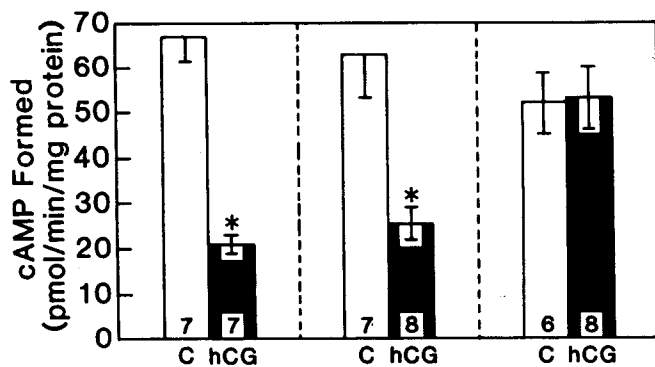

FIG. 2. Uptake of [^{125}I]iodo-hCG by ovarian CL, ectopic CL, and kidney. [^{125}I]Iodo-hCG (50 μCi) was injected iv into four rabbits which had both ectopic and ovarian CL. Two hours after injection, the rabbits were killed, the CL were removed, weighed, and counted for ^{125}I. A small piece of kidney (30 mg) was also removed, weighed, and counted for ^{125}I. Each bar represents the mean ± SEM with n = 4.

hCG-induced desensitization in ovarian vs. ectopic CL

Having demonstrated equal uptake of $[^{125}I]$iodo-hCG by ectopic and ovarian CL, and that there were no differences in serum E_2 concentrations, our next objective was to determine the effects of injecting 75 IU hCG into animals with ectopic CL only or with ectopic and ovarian CL. These data are presented in Fig. 3. As can be seen in Fig. 3A (*left*), LH-stimulated adenylyl cyclase activity in ovarian CL is decreased 69% relative to control after injection of hCG. Similarly, there is a 59% decrease in LH-stimulated adenylyl cyclase activity rel-

A. LH-Stimulated Adenylyl Cyclase
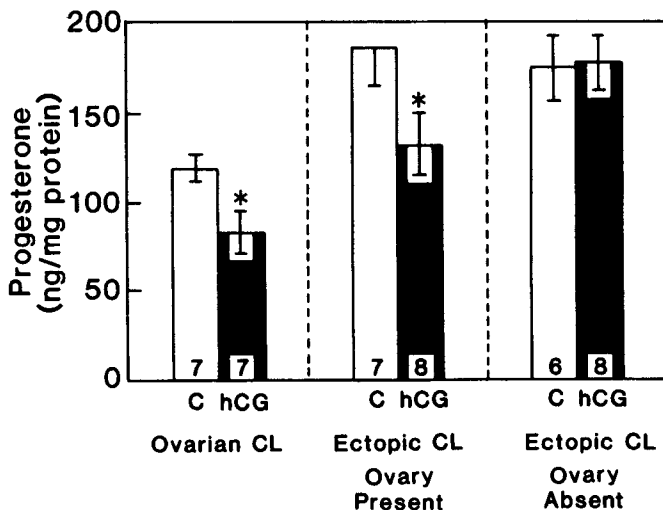

B. Luteal Progesterone

FIG. 3. hCG-induced desensitization of ovarian (*in situ*) vs. ectopic CL as assessed by LH-stimulated adenylyl cyclase activity (A) and luteal progesterone content (B). Rabbits were unilaterally or bilaterally ovariectomized and periovulatory follicles were transplanted underneath the kidney capsule as described in *Materials and Methods*. On day 7 of pseudopregnancy, the rabbits were injected with saline (C) or 75 IU hCG (hCG). The rabbits were killed 24 h later. Each bar represents the mean ± SEM of a group of n animals where n is the number at the bottom of the bar. Bars with asterisks (*) above indicate that the means of the hCG-injected group represented by the bar is significantly different ($P < 0.05$) than the mean of the respective control group.

ative to control in ectopic CL when one ovary remains intact (Fig. 3A; *middle*). In both cases, there are corresponding decreases in luteal progesterone content (40% in ovarian CL and 29% in ectopic CL) relative to control after hCG injection (Fig. 3B; *left* and *middle*). However, ectopic CL which exist in the absence of any other ovarian tissue do not desensitize in response to hCG as judged by the lack of change in either LH-stimulated adenylyl cyclase activity (Fig. 3A; *right*) or luteal progesterone content (Fig. 3B; *right*). Table 1 contains additional adenylyl cyclase data showing that the desensitization obtained upon hCG injection in ovarian CL and in ectopic CL in the presence of an ovary has not only a homologous component, but also a heterologous component as indicated by the fact that adenylyl cyclase responses to isoproterenol and NaF are attenuated as well, albeit to a much lesser extent (22–33%). It can also be seen in Table 1 that there is an elevated basal activity 24 h after hCG injection in the ovarian CL. This is likely due to a continued occupancy of receptors by the injected hCG and thus, the system might be considered to be under hormonal influence. This finding has been reported and discussed in greater detail previously (31). The fact that there is no change in the basal activities of ectopic CL may reflect the absence of more chronic actions of hCG on these tissues.

Effects of hCG injection on $[^{125}I]$iodo-hCG binding in ovarian vs. ectopic CL

Figure 4 contains data from binding assays performed to determine if the loss of responsiveness of the adenylyl cyclase system to LH could, at least in part, be attributed either to decreased levels of available luteal LH/hCG receptors or to a major decrease in the affinity of LH/hCG receptor for hCG. However, as shown in Fig. 4 (*left*), both the total number of LH/hCG receptors and their affinities for hCG did not vary significantly in luteal tissues of saline-injected animals. This indicates that the decrease in desensitization to hCG seen in ectopic CL, especially those of animals without ovaries, could not be

TABLE 1. Adenylyl cyclase activities in homogenates of *in situ* ovarian CL and ectopic CL of rabbits with or without an ovary

CL type	Desensitizing treatment	No.	–	Addition to assay isoproterenol[a]	NaF
Ovarian (*in situ*)[b]	Saline	7	12.9 ± 1.4	47.7 ± 3.9	119.5 ± 9.7
	hCG	7	16.7 ± 0.6[c]	35.1 ± 2.1[d]	91.9 ± 5.7[d]
Ectopic (ovary present)[b]	Saline	7	14.1 ± 1.0	43.2 ± 4.9	93.5 ± 8.2
	hCG	8	12.1 ± 1.2	29.1 ± 3.7[d]	63.8 ± 2.8[d]
Ectopic (ovary absent)[e]	Saline	6	9.1 ± 0.8	31.0 ± 3.2	73.5 ± 8.4
	hCG	8	11.0 ± 0.9	37.7 ± 4.1	81.8 ± 5.1

[a] Data are expressed as mean ± SEM in picomoles of cAMP formed per min/mg protein.
[b] Protocol A of Fig. 1.
[c] hCG group mean greater than saline group mean ($P < 0.05$).
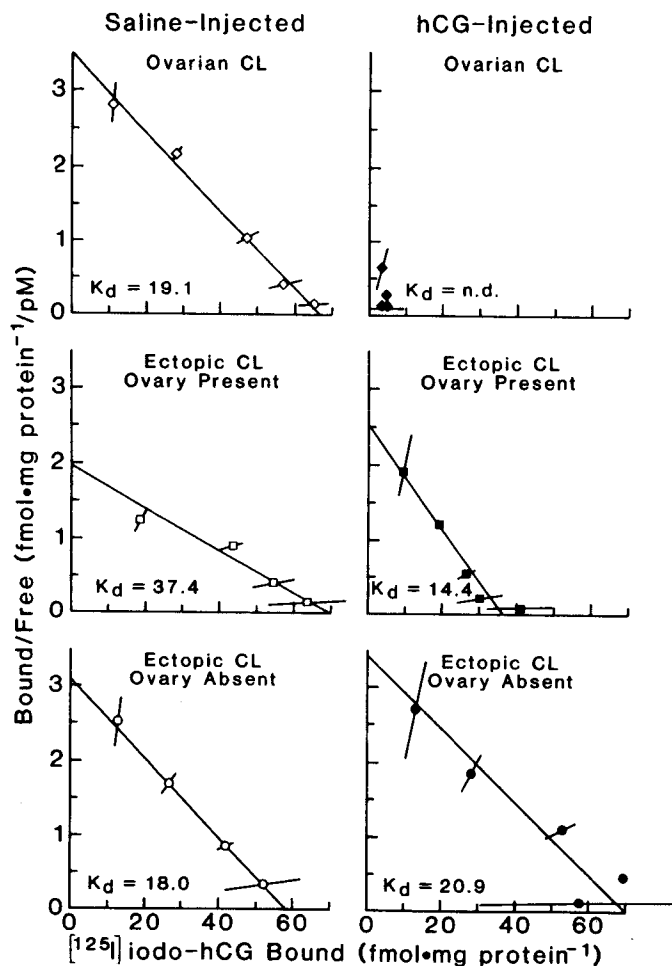[d] hCG group mean less than saline group mean ($P < 0.05$).
[e] Protocol B of Fig. 1.

FIG. 4. Effect of hCG injection on [^{125}I]iodo-hCG binding in ovarian (*in situ*) CL (*top*), ectopic CL with one ovary present (*middle*), and ectopic CL in the absence of the ovaries (*bottom*). Treatment of the rabbits was as described in the legend to Fig. 2 and in *Materials and Methods*. The data shown are Scatchard plots for each of the six treatment groups. Plotted are mean ± SEM of triplicate determinations. The K_d are indicated for each plot (ND, not determined). The B_{max} are the x-intercepts.

accounted for by a decrease in receptor levels and/or receptor affinity. Analysis of available hCG receptor sites in luteal membranes from hCG-treated rabbits (Fig. 4, *right*) showed that, whereas available receptor sites decreased to unmeasurable levels in ovarian CL, they decreased significantly less in ectopic CL of rabbits with one ovary (50% relative to control) and not at all in ectopic CL of nondesensitizing rabbits having no ovary.

Discussion

We have demonstrated that ectopic CL located under the kidney capsule in animals which have an ovary intact respond to a desensitizing dose of hCG in a fashion similar to that found in ovarian CL, *i.e.* there is both a homogous and a heterologous loss of adenylyl cyclase

responsiveness and corresponding decreases in lut progesterone content and available LH/hCG recepto. However, ectopic CL maintained on an E_2-filled Silastic capsule in the basence of other ovarian tissues are resistant to hCG-induced desensitization. Therefore, it is likely that an extraluteal ovarian tissue is required for hCG-induced desensitization of luteal adenylyl cyclase.

Hormone-induced desensitization of adenylyl cyclases can be broken down into two components. First, there is a rapid homologous desensitization which involves the uncoupling of the hormone receptor from the rest of the adenylyl cyclase system (9, 10, 32–36) followed by down-regulation of the receptor (32, 37). The uncoupling phase of this type of desensitization can be mimicked *in vitro* (24, 38, 39) and there are three lines of evidence that suggest that the uncoupling phase of homologous desensitization may involve a phosphorylation of the hormone receptor: 1) a phosphoprotein phosphatase partially purified from rabbit follicles can reverse this type of desensitization (40), 2) Mg and ATP are required for this phase of desensitization and AMP-P(NH)P cannot substitute for ATP (24, 38), and 3) there is no alteration of the guanine nucleotide-binding regulatory (N) component of the adenylyl cyclase system during the homologous phase of desensitization (41) suggesting that the receptor, not N, is altered.

The second phase of hormone-induced desensitizati of luteal adenylyl cyclase is slower in onset and is heterologous in nature (9, 10, 31, 35, 39, 42–45). This phase of desensitization may be cAMP-dependent (39, 42, 44) and appears to be due to or intimately associated with alteration or loss of activity of the regulatory N component of the adenylyl cyclase system (31). It may well be that the alteration of the N component in heterologous desensitization might also be a phosphorylation based on the possible cAMP-dependent nature of the effect.

Our data suggest that ectopic CL in the absence of extraluteal ovarian influences lack one or more factors which may play a role in homologous and heterologous desensitization. Not only is there no apparent loss of LH/hCG receptor in ectopic CL in the absence of other ovarian tissues (despite normal uptake of radiolabeled hCG), but there is no heterologous desensitization of adenylyl cyclase activity resulting from hCG injection either. It is tempting to suggest that these factors are protein kinases. Proof for this requires both actual demonstration that desensitization reactions are causally related to receptor and N component phosphorylation and characterization of these putative kinases and their regulation by receptor occupancy and/or cAMP.

Our data also suggest that extraluteal ovarian tissue produces one or more substances which are required either chronically or acutely for desensitization to occu. The data indicate further that these substances of extra-

eal but ovarian origin can be blood born for they affect ectopic CL as seen by the fact that these CL developed sensitivity to the desensitizing action of hCG in hemiovariectomized rabbits. It should be noted however that the ectopic CL (ovary present) develop less of a sensitivity to the desensitizing action of hCG than normal ovarian counterparts. This is evidenced by the slightly lower level of attenuation of LH-stimulated adenylyl cyclase activity (59% vs. 69% decrease relative to control in ectopic vs. ovarian CL), luteal progesterone content (20% vs. 40% decrease relative to control in ectopic vs. ovarian CL), and available LH/hCG receptor (50% vs. 100% decrease relative to control in ectopic vs. ovarian CL). It would seem therefore that the extraluteal ovarian substance(s) becomes limiting after dilution in, or partial inactivation by, the systemic circulation.

Several questions arise as a consequence of these experiments. First, what is the chemical nature of the extraluteal ovarian influence that confers upon luteal tissue the capacity to desensitize in response to hCG? Second, is the influence always present or does it arise as a consequence of the desensitizing treatment? Third, is the lack of desensitization observed here in ectopic CL of bilaterally ovariectomized rabbits in any way related to the lack of desensitization to FSH observed in preantral ovarian follicles? It is hoped that the ovariectomized rabbit with ectopic CL may serve as a bioassay model for the characterization of the ovarian extraluteal substance that regulates the desensitizing response. This would be a first step towards the answering of the above questions and is the current thrust of our experiments.

Finally, it should be mentioned that hCG injection not only resulted by 24 h in desensitization of the adenylyl cyclase system and loss of available LH/hCG receptors, but also in reduction of luteal progesterone content in ovarian CL. The latter is a clear indication of initiation of a luteolytic process that follows the injection of high levels of hCG into rabbits (46–48). In this context, it is of interest to note that failure of ectopic CL to respond in a desensitizing mode in the absence of other ovarian tissue was accompanied by failure to respond in a luteolytic mode, i.e. by decreasing luteal progesterone content. This is perhaps the first experiments suggesting that the luteolytic effect of hCG may not be a result of the surge in luteal cAMP it causes upon stimulating adenylyl cyclase. Rather, the data suggest that hCG-induced luteolysis may be associated with and/or dependent on desensitization of the adenylyl cyclase system which, in its homologous aspect, is 100% by 48 h after hCG treatments such as given here (9). The implication here is that luteolysis may be dependent upon an extraluteal factor of ovarian origin. Indeed, Miller and Keyes (49) reported the possible involvement of such a factor in luteolysis of ectopic CL. Thus, further investigations into the nature

of the ovarian factor(s) required for development of a desensitizing response of the luteal adenylyl cyclase upon treatment with hCG may, perhaps, also lead to new insights into regulatory aspects of CL regression.

References

1. Hilliard J, Spies HG, Lucas L, Sawyer, CH 1968 Effect of prolactin on progestin release and cholesterol storage by rabbit ovarian interstitium. Endocrinology 82:122
2. Hilliard J, Eaton Jr LW 1971 Estradiol-17β, progesterone and 20 α-hydroxypregn-4-ene-3-one in rabbit ovarian venous plasma. II. From mating through implantation. Endocrinology 89:522
3. Younglai EV 1972 Effect of mating on follicular fluid steroids in the rabbit. J Reprod Fertil 30:157
4. Hilliard J, Scaramuzzi RJ, Pang CN, Penardi R, Saywer CH 1974 Testosterone secretion by the rabbit ovary in vivo. Endocrinology 94:267
5. Butcher RL, Collins WE, Fugo NW 1974 Plasma concentrations of LH, FSH, prolactin, progesterone, and estradiol-17β throughout the 4-day estrous cycle of the rat. Endocrinology 94:1704
6. Hillensjo T, Bauminger S, Ahren K 1976 Effect of luteinizing hormone on the pattern of steroid production by preovulatory follicles of pregnant mare's serum gonadotropin-injected immature rats. Endocrinology 99:996
7. Katz Y, Armstrong DT 1976 Inhibition of ovarian estradiol-17β secretion by luteinizing hormone in prepubertal, pregnant mare serum-treated rats. Endocrinology 99:1442
8. Marsh JM, Mills TM, LeMaire WJ 1973 Preovulatory changes in the synthesis of cyclic AMP by rabbit Graafian follicles. Biochim Biophys Acta 304:197
9. Hunzicker-Dunn M, Birnbaumer L 1976 Adenylyl cyclase activities in ovarian tissues. II. Regulation of responsiveness to LH, FSH, and PGE₁ in the rabbit. Endocrinology 99:185
10. Hunzicker-Dunn M, Birnbaumer L 1976 Adenylyl cyclase activities in ovarian tissues. IV. Gonadotropin-induced desensitization of the luteal adenylyl cyclase throughout pregnancy and pseudopregnancy in the rabbit and the rat. Endocrinology 99:211
11. Bruce HM 1959 An exteroceptive block to pregnancy in the mouse. Nature 184:105
12. Chapman VM, Desjardins C, and Whitten WK 1970 Pregnancy block in mice: changes in pituitary LH and LTH and plasma progestin levels. J Reprod Fertil 21:333
13. Birnbaumer L, Kirchick HJ 1983 Regulation of gonadotropic action: the molecular mechanisms of gonadotropin-induced activation of ovarian adenylyl cyclases. In: Greenwald G, Terranova P (eds) Regulation of Ovarian Function. Raven Press, New York, pp 287–310
14. Jonassen JA, Bose K, Richards JS 1982 Enhancement and desensitization of hormone-responsive adenylate cyclase in granulosa cells of preantral and antral ovarian follicles: effects of estradiol and follicle-stimulating hormone. Endocrinology 111:74
15. Walseth TF, Johnson RA 1979 The enzymatic preparation of [alpha-³²P]nucleoside triphosphates, cyclic [³²P]AMP and cyclic [³²P]GMP. Biochim Biophys Acta 562:11
16. Birnbaumer L, Torres HN, Flawia MM, Fricke RF 1979 Improved methods for determination of guanylyl cyclase activity and synthesis of [alpha-³²P]GTP. Anal Biochem 93:124
17. Legan SJ, Coon GA, Karsch FJ 1975 Role of estrogen as initiator of daily LH surges in the ovariectomized rat. Endocrinology 96:50
18. Gospodarowicz D 1973 Properties of the luteinizing hormone-receptor of isolated bovine corpus luteum plasma membranes. J Biol Chem 248:5042
19. Abramowitz J, Iyengar R, Birnbaumer L 1982 Guanine nucleotide and magnesium ion regulation of the interaction of gonadotropic and β-adrenergic receptors with their hormones: a comparative study using a single membrane system. Endocrinology 110:336
20. Keyes PL, Armstrong DT 1969 Development of corpora lutea from follicles autotransplanted under the kidney capsule in rabbits. Endocrinology 85:423

21. Cohen RP 1957 Suspending media for animal tissues. In: Umbriett WW, Burris RH, Stauffer JF (eds) Manometric Techniques. Burges, Minneapolis, p 149

22. Birnbaumer L, Yang P-Ch, Hunzicker-Dunn M, Bockaert J, Duran JM 1976 Adenylyl cyclase activities in ovarian tissues. I. Homogenization and conditions of assay in Graafian follicles and corpora lutea of rabbits, rats, and pigs: regulation by ATP and some comparative properties. Endocrinology 99:163

23. Salomon Y, Londos C, Rodbell M 1974 A highly sensitive adenylate cyclase assay. Anal Biochem 58:541

24. Bockaert J, Hunzicker-Dunn M, Birnbaumer L 1976 Hormone-stimulated desensitization of hormone-dependent adenylyl cyclase. J Biol Chem 251:2653

25. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ 1951 Protein measurement with the Folin phenol reagent. J Biol Chem 193:265

26. Iyengar R, Abramowitz J, Bordelon-Riser ME, Blume AJ, Birnbaumer L 1980 Regulation of hormone-receptor coupling to adenylyl cyclases: effects of GTP and GDP. J Biol Chem 255:10312

27. Kirchick HJ, Birnbaumer L 1981 Effects of withdrawal of exogenous estradiol from pseudopregnant rabbits: transient nature of loss of luteal function and reversal of estradiol-induced suppression of luteinizing hormone-responseive adenylyl cyclase. Endocrinology 109:2129

28. Goodman RL, Legan SJ, Ryan KD, Foster DL, Karsch FJ 1980 Two effects of estradiol that normally contribute to the control of tonic LH secretion in the ewe. Biol Reprod 23:415

29. Midgley Jr AR, Niswender GD, Rebar RW 1969 Principles for the assessment of the reliability of radioimmunoassay methods (precision, accuracy, sensitivity, specificity). Acta Endocrinol [Suppl] (Copenh) 142:247

30. Duddleson WG, Midgley Jr AR, Niswender GD 1972 Computer program sequence for analysis and summary of radioimmunoassay data. Comput Biomed Res 5:205

31. Kirchick HJ, Iyengar R, Birnbaumer L 1983 Human Chorionic Gonadotropin-induced heterologous desensitization of adenylyl cyclase from highly luteinized rat ovaries: attenuation of regulatory N component activity. Endocrinology 113:1638

32. Marsh JM, Mills TM, LeMaire WJ 1972 Cyclic AMP synthesis in the rabbit Graafian follicle and the effect of luteinizing hormone. Biochim Biophys Acta 273:389

33. Hsueh AJW, Dufau ML, Catt KJ 1977 Gonadotropin-induced regulation of luteinizing hormone receptors and desensitization of testicular 3',5'-cyclic AMP and testosterone responses. Proc Natl Acad Sci USA 74:592

34. Lamprecht SA, Zor U, Salomon Y, Koch Y, Ahren K, Lindner HR 1977 Mechanism of hormonally induced refractoriness of ovarian adenylate cyclase to luteinizing hormone and prostaglandin E_2. J Cyclic Nucleotide Res 3:69

35. Harwood JP, Richert ND, Dufau ML, Catt KJ 1980 Gonadotropin-

36. Hunzicker-Dunn M 1981 Rabbit follicular adenylyl cyclase activ. II. Gonadotropin-induced desensitization in granulosa cells and follicle shells. Biol Reprod 24:279

37. Suter DE, Fletcher PW, Sluss PM, Reichert Jr LE, Niswender GD 1980 Alterations in the number of ovine luteal receptors for LH and progesterone secretion induced by homologous hormone. Biol Reprod 22:205

38. Iyengar R, Mintz PW, Swartz TL, Birnbaumer L 1980 Divalent cation-induced desensitization of glucagon stimulable adenylyl cyclase in rat liver plasma membranes: GTP-dependent stimulation by glucagon. J Biol Chem 255:11875

39. Hunzicker-Dunn M, Birnbaumer L 1981 Studies on the mechanism of LH-induced desensitization of the rabbit follicular adenylyl cyclase system in vitro. Endocrinology 109:345

40. Hunzicker-Dunn M, Derda D, Jungmann RA, Birnbaumer L 1979 Resensitization of the desensitized follicular adenylyl cyclase system to LH. Endocrinology 104:1785

41. Iyengar R, Bhat MK, Riser ME, Birnbaumer L 1981 Receptor-specific desensitization of the S49 lymphoma cell adenylyl cyclase. Unaltered behavior of the regulatory component. J Biol Chem 256:4810

42. Su YF, Cubeddu XL, Perkins JP 1976 Regulation of adenosine 3':5'-monophosphate content in human astrocytoma cells: desensitization to catecholamines and prostaglandins. J Cyclic Nucleotide Res 2:257

43. Hunzicker-Dunn M, Day SL, Abramowitz J, Birnbaumer L 1979 Ovarian responses of pregnant mare serum gonadotropin and human chorionic gonadotropin-primed rats: desensitization, luteolytic and ovulatory effects of a single dose of human chorionic gonadotropin. Endocrinology 105:442

44. Nickols GA, Brooker G 1979 Induction of refractoriness to isoproternol by prior treatment of C6-213 rat astrocytoma cells with cholera toxin. J Cyclic Nucleotide Res 5:435

45. Kirchick HJ, Birnbaumer L 1981 Prostaglandins do not appear play a role in hCG-induced regression or desensitization of rab. corpora lutea. Biol Reprod 24:1006

46. Stormshak F, Casida LE 1964 Effect of gonadotropins on corpora lutea of pseudopregnant rabbits. Endocrinology 75:321

47. Stormshak F, Casida LE 1965 Effects of LH and ovarian hormones in corpora lutea of pseudopregnant and pregnant rabbits. Endocrinology 77:337

48. Spies HG, Coon LL, Gier HT 1966 Luteolytic effect of LH and hCG on the corpora lutea of pseudopregnant rabbits. Endocrinology 78:67

49. Miller JB, Keyes PL 1976 A mechanism for regression of the rabbit corpus luteum: uterine-induced loss of luteal responsiveness to 17β-estradiol. Biol Reprod 15:511